The Arrayer

increasing X

increasing Y

increasing rows

Console home
(0, 0, 320)

← highest Z value

GUI home
(280,000, 0, 320)

1 3
2 4

increasing columns

Slides

				#80	#41	#40	#1
							#2
							#3

| | | | | #61 | #60 | #21 | #20 |

robotic arm

A1

Source plate

H20 wash

house vacuum

coordinates are in encoder units

1 encoder unit (EU) = 2.5 μm in X or Y dimension

1 EU = 5 μM in Z dimension

The Slide

```
         (30,000, 0)                              (0, 0)
    ↑  ┌──────────────────────────────────────────────┐
       │                    center                     │
25 mm  │               (15,000, 5,000)                 │
(10.000 EU)                                            │
    ↓  │ (30,000, 10,000)              (0, 10,000)     │
       └──────────────────────────────────────────────┘
       ←─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─→
                75 mm = 30,000 EU
```
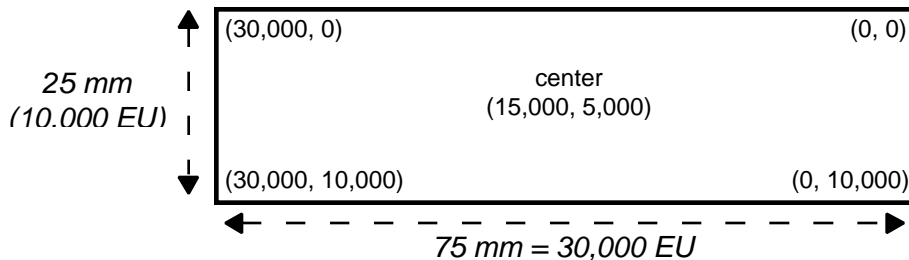
Slide preparation:
- slides can be stored for up to 6 months
- slides must be used immediately after activation
- use unsilanized slides for test spotting

Slide position:
- slide should lie flat on arrayer
- slides should be aligned to top of column and left of row starting with far right column
- to secure slides in position on arrayer, pipette 5µl ddH2O on bottom of each slide before placing on arrayer

Slide coordinate system:
- use in GUI for determining where to start arraying (*Arrayer position X & Y*)

The Source Plate
as oriented on plate holder on arrayer

96 well plate

384 well plate
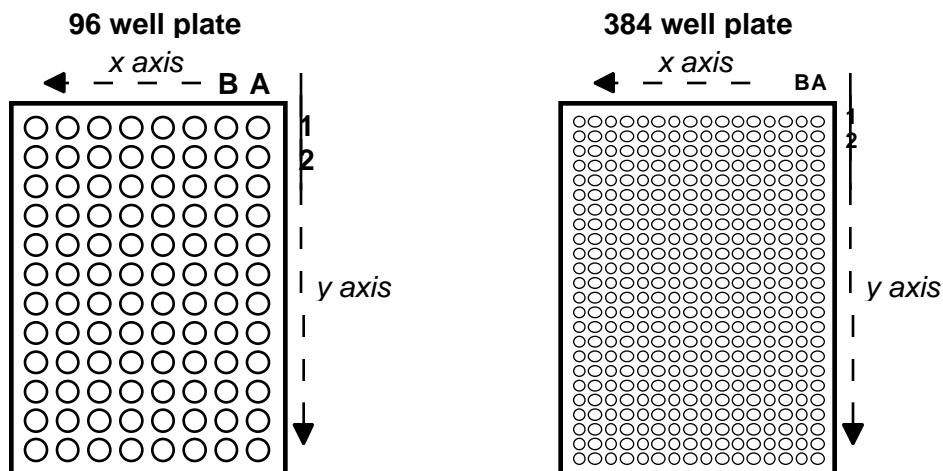
Source plate position:
- make sure plate is centered on the plate holder
        (a propped up plate could damage an arrayer pin!)
- A1 should be in the upper right hand corner of plate holder
- only open 1/3 of foil at a time on the plate during arraying to prevent evaporation
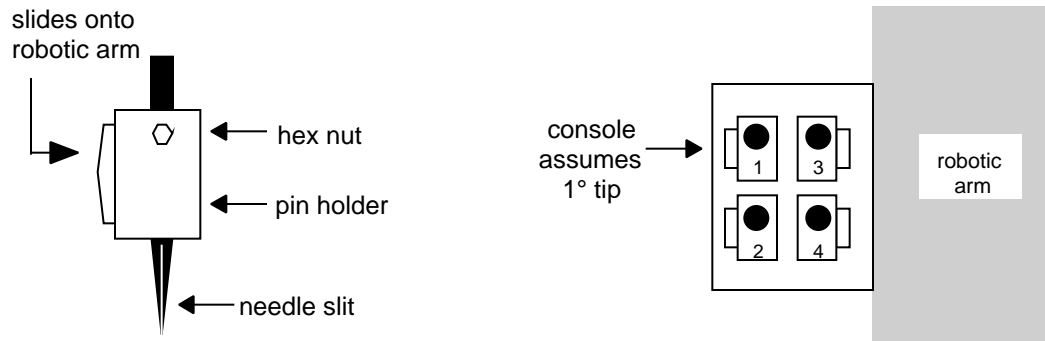
<u>Arrayer Startup Protocol</u>

<u>Starting the instrument:</u>

1. Log onto computer   • arrayer power should be off

2. Start "*Arrayer 2.0 C*"   • results in a console window & gui window

3. On console hit "enter"   • results in "%" prompt

4. % *tellE*   • checks if computer is oriented
    - if response is "0, 0, 0" then continue
    - if response is not, exit software & restart computer
    - if problem persists, call RickNorgren

    650-813-1181

5. Turn power strip on & check for green status lights

6. Turn vacuum on, fill ddH$_2$0 tank, and empty water waste

7. Cover vacuum holes briefly   • should flush hoses

8. % *tellE*   • response should be "0, 0, 0"
    - if not, go back to step #2

9. % homeAll   • moves arrayer to 0, 0, 320 (upper left corner)

10. % washTip   • washes tip

                                     • in GUI, can also hit "Wash" button

- Inspect pin under light microscope

<u>Arrayer Pins</u>

slides onto
robotic arm

hex nut

pin holder

needle slit

console
assumes
1° tip

robotic
arm

<u>Pin quality:</u>
- use light microscope to examine pin tip
- pin tip should not be dirty or damaged; ie, tip must not be occluded or flattened due to impact)
- evaluate experimentally by spotting targets on non-silanized slide
      (criteria = spot diameter, presence of halo, and spacing between spots)

<u>Putting pin in holder:</u>
- slit should be parallel to hex nut (thus it is parallel to wash)
- use flat end of pin head and/or light microscope to judge orientation
- tighten hex-nut with pin in correct position (note: don't ever loosen nut all the way)

<u>Pin holder:</u>
- should go into robotic arm with Pin head @ lowest Y value (see right diagram)
- pin holder should move up and down smoothly in robotic arm
      (if not, then the pin is easily damaged by impact on slide!)
- hex-nut should always be at top of pin holder (see left diagram)

<u>Cautions:</u>
- do not bend or damage pin tips when ....
      i. putting them in pin holder
      ii. putting pin holder into the robotic arm

<u>Pin positions:</u>
   Console --> Pin#1 is reference tip
   GUI =>   Pin #1 is reference tip if setting is 1 pin
                     Pin #3 is reference pin if setting is 4 pin
                           (this pin will go to A1 for first sample)

13. Begin test spotting

- Use unsilanized slides for test spotting
- Choose 10 random clones to be tested from each plate.
- Use the % arraySample console command to spot each test clone
  - between 20 and 200 times (depending on number arrays to be made)

Example:

% arraySample 1 3 5 24000 3200 80 2 84 ——— EU spacing between spots

# of columns, rows of spots

X,Y coordinate of first spot

• makes an 80X2 array of clone in well A3 on slide 5, with upper left spot at (24000,3200)

slide 5, column 1

well A 3

14. Inspect pin again under light microscope

15. DAPI test slide
- Pipette 15ul of 1 or 2 µg/ml DAPI dilution onto test array
  (DAPI is stored in the door of 1745 freezer in a box marked "DAPI")
- Apply cover slip and let sit for 5-10 minutes
- Dunk slide in copelin jar containing ddH2O until cover slip falls off
- Shake slide to remove excess water and swipe the back with a kimwipe
- Image slide and put a copy of the image in the Array Binder in 1653.

16. Prepare a GUI worksheet containing the following information:

Source

    Plate type: __   384- or 96-well plate (384   is the default)

    Total # samples: __ # of samples the plate contains

Slides

    # of Rows: __   # of rows of slides on the arrayer

    # of Columns: __   # of columns of slides on the arrayer

Array

```
Number of tips: __       1, 2, or 4 pins used for arraying
Sub-array: __            1, 2, or 4 sub-arrays, same as the number of tips
                         used
# Sub-array rows: __     # of rows the portion of the array made by this
                         plate will have
# Sub-array columns: __     # of columns the portion of the array made
                            by this plate will have
Array element spacing: __ Spot center-to-center spacing (microns)
X position from right side of slide: __   This is the slide position of
Y position from the top of slide: __      the first spot from this
                                          plate (milimeters)
```

17. Open "Change Parameters" under "File" in the GUI window

This opens a new window titled "Input Arrayer Parameters". Adjust the parameters according to the GUI worksheet and hit "Apply". You will notice that the initial GUI window now displays the new parameters. Note that this window displays "Number of Slides", "Number of Subarrays", and "Number of Subarray Rows" even though these parameters are not entered in by the user. They are calculated by the software according to the other parameters. Make sure that what has been calculated matches the GUI worksheet.

18. Hit "Array"

Note: hit the array button ONLY ONCE for each plate arrayed

- To pause the robot, first click in the pause box ONLY ONCE and wait for the robot to finish its last task (the checkmark in the box will not appear until the last task has been completed)
- To restart, click the pause box once.

- When you hit "Array" the robot will wash the pins once and then go to the GUI "Home" position. You must hit "Enter" in the Dialogue box before the arraying actually begins.

- After each plate is arrayed, check the pin under the light microscope, empty the wash water waste, make sure there is enough water in the water pump container and check the humidity.

- Change the appropriate parameters before each new plate is arrayed.

19. When arraying is complete:

- Place slides in the 37 degree humid chamber for 15 - 30 minutes and then into a slide box. Slightly prop open the box top and let slides dry overnight.

- Take any direct labeled target out of the sample plates and save in a cardboard box in a -20 degree freezer.

- If these samples are to be used again, lyophilize, seal, and store in -20 degree freezer.

- Save all material on the console to a text file, print, and add to the Arrayer Notebook in 1653.

20. Shutting down the arrayer

- %quit to close console window
- close GUI window
- take the pump out of its container and allow the hoses to clear out any water left in them
- turn off the power strip

- turn off the vacuum

- empty the wash water waste

- cover the arrayer

- turn off any humidifiers or hotplates

GUI Worksheet

Source			
Plate Name			
No. array elements			
Plate type			
Slides			
No. of Rows			
No. of Columns			
Array			
No. print tips			
No. subarrays			
No. subarray rows			
No. subarray columns			
Array element spacing(μm)			
X Position (mm)			
Y Position (mm)			
Replicate offset			

NOTES:

Arrayer parameters file info

- File name = LLNL parameters.tcl

- File that defines coordinates & constants (referenced as globals)

- Active file is in   **c:_____/_____/**LLNL parameters.tcl

- Unmodified file is in **c:_____/_____/**LLNL parameters.tcl

- Every time this file is changed, one must exit software (arrayer 2.0 c) and restart to reload these parameters.
- Parameters that might vary are in italics & Bold

wash station constants & parameters

set wSpacing 7200	# spacing between wash + dry
set washPosX 281415	# X coordinates for wash
set washPosY 183910	# Y coordinates for wash
set ZWashDownPos –2030	# Z coordinate for wash
set ZdryDownPos –2030	# Z coordinate for drying
set washTime ***12000***	# gives wash time in ms (affects exp. Time)
set vacTime ***8000***	# gives dry time in ms (affects exp. Time)

#384-well plate parameters (default)

set firstPosX(384) 294215	# X coordinate for well A1 in 384 plate
set firstPosY(384) 103910	# Y coordinate for well A1 in 384 plate
set ZplateDownPos(384) ***–2250***	# Z coordinate for well sampling (optimize)
set wellSpacing(384) 1800	# well spacing for 384 plate in X & Y dimension
set plateDwellTime ***5000***	# time pin samples from any plate

#96-well plate parameters

- relative to position of wells in 384-well plate

set firstPosX(96) [expr $firstPosX(384) –900]   # this is the position of well A1 in a set
firstPosY(96) [expr $firstPosY(384) –900]   96 well plate relative to A1
                                                               in 384 well plate

X = -900

A1   Y = +900

A1

384-well
x, y position
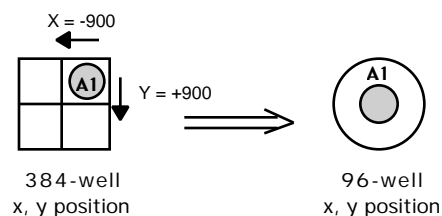
96-well
x, y position

Special statements for arraying 96 samples out of 384-well plate

- overrides 96-well plate parameters above, allows us to use 96-well format in a 384 well plate
- comment these 4 statements out if using a 96-well plate
- comment these 4 statements out if using 384 samples in 384-well plate

set firstPosX(96) [expr $firstPosX(384)] # overrides the "96-well plate parameters
set firstPosY(96) [expr $firstPosY(384)]   so there is no offset at shown above

set ZplateDownPos(96) *–2080*   # overrides previous command (Note that the initial Z down
                                     position was so low the pin was scratching the slide)

set wellSpacing(96) [expr $wellSpacing(384) * 2]   # sets well spacing to double that
                                                     if using all 384 wells

```
  C A
○●○● 1
○○○○
○●○● 3
○●○● 5
```
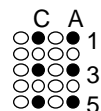
#default plate as 384

set array(plateType) 384   # change this if default plate changes

#default number of tips as 1

set array(numTips) 1   # defines the number of printing tips

#setting double print option

- indicates status of replicate spotting
- make sure offset is sufficient to prevent overlap between bottom rows of superior array and top rows of inferior array

set printDoubleOption *1*   # 1 means replicate
                                          # change to 0 if no replicate

set printDoubleSpacing *1200*   # defines the vertical offset for replicate spot
                                          # this depends on number of rows

#definitions of relevant array parameters
- in Z axis, 1 EU = 5 µm and for X & Y, 1 EU = 2.5 µm

set arrayRelPosX *8000*   # defines upper left corner on slide
set arrayRelPosY *2000*   to begin the array

```
(30,000, 0)                      (0, 0)
                    ?


(30,000, 10,000)                 (0, 10,000)
```
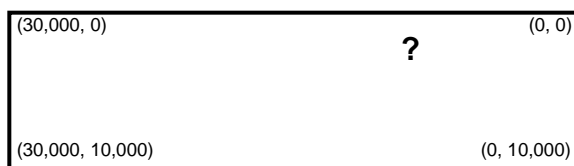
```
set slide1EdgeX 255275          # defines position on arrayer of slide #1(?)
set slide1EdgeY –2450

set slideSpacing 33020          # defines slide-to-slide EU spacing in X direction

set slideWidth 10040            # defines slide-to-slide EU spacing in Y direction

# Zprinting parameters
set ZprintLevel –2270           # defines pin height when spotting a slide
                                # old level = -2290

set Zdelta 200                  # lift in Z before moving pin

setZUpPos 320                   # maximum absolute Z value

set beforePrintDelay 1          # ???

set afterPrintDelay 1           # ???

# machine parameter
set mmToEncoder 400             # defines 1 mm = 400 EU or 1 EU = 2.5 µm

# misc. parameters
set xRestPos 280000             # define X & Y  "home" dimensions
set yRestPos 0

set arrInfo(delay) 1            # ???
```

Arrayer Console Commands

% tellE
- checks if computer is oriented in x, y, & z
- if so, then console says "0, 0, 0"
- if not, then console indicates possible x, y, z errors

% homeAll
- moves pins to 0, 0, 320 (x, y, z)

% where
- indicates x, y, z position of pin #1

% washTip
- washes and dries tip once

% set array (plateType) 96
- sets source plate to 96 well (consol default = 384 well plate)
- if successful, then console says "96"

% listSampleList
- gives list of source plate wells that will be used

% moveToSlide #
- moves pin#1 to position 0, 0 of designated slide (0, 0 = upper right corner of slide)

% moveToSample *row# column#*
- moves pin #1 to designated row & column of source plate

% move Z #
- moves Z indicated amount from current position

% move aZ #
- moves Z to absolute value indicated

% startZSlowSpeed
- allows user to move up or down the Z axis in increments

% arraySample 1 3 5 24000 3200 80 2 84 —— EU spacing between spots

# of columns, rows of spots

X,Y coordinate of first spot

slide 5, column 1
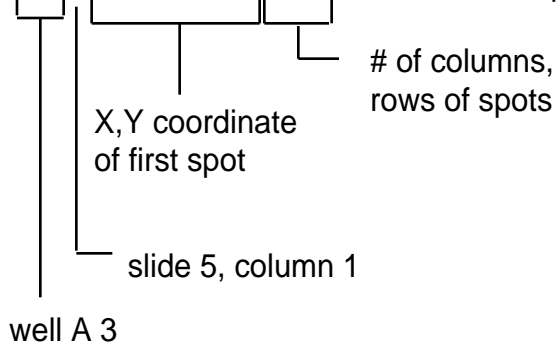
well A 3

- makes an 80X2 array of clone in well A3 on slide 5, with upper left spot at (24000,3200)

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